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Induction of tenascin in cancer cells by interactions with embryonic mesenchyme mediated by a diffusible factor

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SUMMARY

Human cancer cell lines A431 and MCF7, which do not produce tenascin (TN) in vitro, were found to produce TN when injected into nude mice or co-cultured with the embryonic mesenchyme. The TN expression in the developing A431 solid tumor was demonstrated by immunohistochemistory and by in situ hybridization. Human TN was detected in culture media by western blot analysis using human specific monoclonal antibody (RCB-1). During tumorigenesis, in the early stage, mouse TN was actively induced and deposited in the peri- and intertumor spaces surrounding the developing tumor. Two days later, TN derived from human epithelial cancer cells was induced and mainly deposited in the intertumor basement membrane. After this stage, tumor cells were actively producing TN. On the other hand, TN induction in non TN-producing cells, such as A431 and MCF7 cell lines, was also observed in vitro. Although cell lines such as NIH-3T3, φ2 , STO, 2H6, 3E5 and CMT315, had no effect on the TN induction, primary cultured embryonic mesenchyme effectively stimulated the TN expression in the cancer cell lines. This mesenchymal effect decreased with age and was entirely lost postnatally. Furthermore, conditioned media from these embryonic mesenchymes could reproduce the same effects on TN induction as observed in the co-culture study.

In conclusion, these findings suggest that TN induction in epithelial cancer cells may depend on interactions with the surrounding environment, that these interactions may be mediated by a soluble factor(s) derived from the surrounding mesenchyme and that the TN induction observed in the tumorigenesis may reflect histogenesis during the embryonic period.

Key words: tenascin, human cancer cells, extracellular matrix, cell-cell interaction, embryonic mesenchyme

INTRODUCTION

Tenascin (TN), an extracellular matrix (ECM) glycoprotein, is characterized by its nonuniversal distribution in the tissue. Unlike other ECMs, TN appears in limited areas at specific times, such as in the mesenchyme surrounding growing epithelia, fetal epithelia or malignant tissues (Bourdon et al., 1983; Chiquet-Ehrismann et al., 1986; Inaguma et al., 1988; Mackie et al., 1989; Natali et al., 1990) and in the connective tissue of healing wounds (Mackie et al., 1988). By virtue of its spatio-temporally restricted expression, TN has aroused the interest of many researchers who suspect that it is an essential molecule in epithelial-mesenchymal interactions. During the last few years, cDNAs of chick (Jones et al., 1989; Pearson et al., 1988), human (Nies et al., 1991; Siri et al., 1991), mouse (Saga et al., 1991; Weller et al., 1991) and porcine (Nishi et al., 1991) TNs have been cloned, and amino acid sequences determined. There were several isoforms derived from post-transcriptional splicing of precursor mRNA, and from post-translational modification of the polypeptides by carbohydrates. A variety of monoclonal and polyclonal antibodies were also produced. Using these cDNAs, polypeptides and antibodies as probes, many experiments have been attempted in order to demonstrate the biological functions of TN in vivo (reviewed by Chiquet-Ehrismann, 1990). Immunohistochemical studies have revealed that TN appears in the stroma during oncofetal development and perturbed tissue interaction (Sakakura and Kusano, 1991).

The question of what induces TN in the tissue is extremely important. Several findings have suggested that TN synthesis is induced in the stroma as a result of interactions with either embryonic or neoplastic epithelium. If embryonic mammary epithelium is cultured on mammary fat pad precursor tissue, the epithelium undergoes mammary gland morphogenesis. A halo of dense feeder cells is formed around the epithelium where TN accumulates (Inaguma et al., 1988). Co-culture between oral mesenchyme and dental epithelium deposited TN in the matrix at the contact site of these two tissues (Vainio et al., 1989). In the kidney, TN appears in the mesenchyme surrounding the differentiating epithelium (Aufderheide et al., 1987). Gut mesenchyme expresses TN when interacting with the developing epithelium or cultured epithelial cells (Aufder-

heide and Ekblom, 1988). Although these studies strongly suggest that TN expression in the mesenchyme is induced by the epithelium, the cellular source of TN production still remains unclear.

Recently two studies by in situ hybridization have demonstrated that the first site of TN synthesis during lung and feather organogenesis is the epithelium. In 6-day chick embryonic lungs (Koch et al., 1991) and in early feather morphogenesis (Tucker, 1991), TN mRNA was expressed in actively growing epithelial buds and in the epidermis but not in the underlying dense mesenchyme. The accumulation of immunoreactive TN was observed in the basement membrane and underlying mesenchyme but not in the epithelium. These results suggest that TN can be produced and secreted by the epithelium, but should be deposited in the stroma. A question arises as to whether this is also the case during cancer development. Investigating the dynamic change of TN expression in the tissue is important, particularly for the studies of possible effects on malignant cell growth and invasion. The present study was designed to determine the cellular source of TN induced by epithelial-mesenchymal interactions by both in vitro and in vivo systems. We examined TN expression in the solid tumor formed by human epithelial cancer cells in nude mice and in these cells co-cultured with feeder cells or with their conditioned media, using human TN specific antibody.

MATERIALS AND METHODS

Animals

BALB/c nude mice were purchased from CLEA, Tokyo, Japan and maintained in the animal facility in RIKEN, Tsukuba Life Science Center, Tsukuba, Japan. They were used as hosts for the cancer cell transplantation experiments.

Cells

In this study, the following cell lines were used: A431 (human skin epidermoid carcinoma cell line), HT1080 (human fibrosarcoma), NIH-3T3, ϕ2 and STO (mouse fibroblast cell lines) purchased from ATCC; MCF7, a kind gift of Drs Lippman and Dickson, Lombardi Cancer Research Center, DC; 2H6 (fibroblast cell line) and 3E5 (epithelial cell line) established from GR mouse mammary tumor in our laboratory and CMT315 (epithelial cell line) from C3H mouse mammary tumor (Inaguma et al., 1988). Dermis and mammary fat pads were obtained from day-14, -16 and -18 embryos, and new born and adult mice. Dermal tissue fragments of day-9 chick embryos were also used. These tissues were minced and trypsinized with phosphate buffered saline (PBS) containing 0.25% trypsin and 0.25% EDTA (Gibco, Grand Island) for 30 min at 37˚C. After inactivation of enzymatic activity by the addition of culture medium containing 10% fetal calf serum, dissociated cells were collected by centrifugation at 1500 revs per min for 5 min and resuspended in Dulbecco's modified Eagle minimum essential medium (Nissui, Tokyo) supplemented with 10% fetal calf serum (IBL, Fujioka).

Co-culture

NIH-3T3, ϕ2, STO, 2H6, 3E5 and CMT315 cells and mouse and chick mesenchymal cells were used as feeder cells. Each cell line was seeded on culture dishes (Falcon, no. 3003, New Jersey) at a density of 1×10^6 cells/ml and incubated for two days. After these cells had become confluent, A431 or MCF7 cells were plated on these feeder cells at the same density and cultured for three days. To test for soluble factor(s) from feeder cells, A431 or MCF7 cells were cultured for three days in the presence of 50% conditioned media from each feeder cultured for four days.

Tumors

5×10^6 A431 cells were subcutaneously transplanted underneath the skin of each BALB/c nude mouse. The developing tumors were harvested at 1, 3, 5, 10 and 30 days after transplantation and fixed with 10% neutralized buffered formalin in 0.1 M sodium phosphate buffer, pH 7.2. After fixation, tissues were rinsed, dehydrated and embedded in polyester wax (BDH, Poole, UK) according to the method described previously (Kusakabe et al., 1988). The same numbers of cells were also injected into adult mammary fat pad, testis, liver, kidney, lung, spleen and skeletal muscle. The tumors were obtained at 5 days after injection.

Antibodies

Polyclonal rabbit anti-human TN antibody and rat monoclonal anti-human TN antibody, RCB-1 (Oike et al., 1990) were used. Polyclonal antibody was developed by immunizing rabbit with purified TN derived from cultured human fibroblasts in the conventional way. Immunization was carried out every two weeks. The rabbit was bled and the serum was tested for antibody. Since this antiserum contained the antibody which can recognize fibronectin molecules, any contaminating antibody was adsorbed by a fibronectin liganded agarose (PSL, Paesel). For the staining of basement membrane, anti-collagen IV polyclonal antibody (Chemicon, St. el segundo) and anti-laminin polyclonal antibody, a kind gift of Dr. Chiquet, FMI, Basel, were used.

Immunohistochemistry

Sections were stained with either rabbit polyclonal antibody or RCB-1 by enzyme immunohistochemistry. Sections were pretreated with avidin D and biotin blocking kit (Vector, Burlingame). After washing with PBS, they were incubated with PBS containing 1% bovine serum albumin (BSA) (Sigma, St. Louis) and 5% normal goat serum (block A) and subsequently incubated overnight with either rabbit polyclonal antibody or RCB-1 at room temperature (RT). After washing and incubating with ABC kit (Vector, Burlingame), they were developed with 200 ml of PBS containing 40 mg of 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Dojindo, Kumamoto) and 50 μl of H_2O_2. Samples were observed under a microscope (Olympus, Tokyo). For double staining of TN and collagen IV or laminin, sections were firstly incubated with block A for 30 min and subsequently incubated with RCB-1 and either anti-collagen IV or anti-laminin overnight at RT. After washing, they were again incubated for 1 h with the mixture of FITC-labeled anti-rat IgG (Zymed, San Francisco) and rhodamine-labeled anti-rabbit IgG (Tago, Burlingame). Samples were observed by confocal laser scanning microscopy (Carl Zeiss, West Germany).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

The content of TN in the conditioned media was analyzed by western blotting. TN molecules in the conditioned media were collected by 40% ammonium sulfate precipitation. After centrifugation at 10,000 revs per min for 30 min, each pellet was dissolved in 2 M Tris-HCl, pH 7.2. These solutions were used as samples after brief centrifugation to remove the insoluble particles. For western blot analysis these samples were mixed with an equal volume of Seprasol II w/BME (Enprotech), heat-denatured for 2 min in boiling water and applied to 4%-15% polyacrylamide gradient gels (Daiichi, Tokyo). SDS-PAGE was performed accord-

ing to Laemmli's method (1970). After electrophoresis, the separated proteins were electrically transferred onto the immobilon transfer membrane (Millipore, Tokyo) in SDS-free Tris-glycine (0.025 M Tris, 0.192 M glycine, pH 8.3) buffer containing 10% methanol for at least 5 h. After blotting, the membrane was incubated with the blocking solution composed of 50% (v/v) of 50 mM Tris-HCl-saline (TBS), pH 7.6, containing 5% normal goat serum, 1% BSA and 50% Block Ase (UK-B25, Dainippon, Osaka), block B, to block the non-specific binding site of the membrane. Subsequently, the first antibody (final concentration, 10 μg/ml) was added to the blocking solution and incubated overnight at RT. After thorough rinsing with ice-cold TBS, the membrane was incubated for 2 h with anti-rat IgG antibody (10 μg/ml, Zymed, San Francisco) diluted with the block B, rinsed in TBS, then incubated for 1 h with rat peroxidase anti-peroxidase complexes (Zymed, San Francisco) diluted 1 : 500. The enzymatic activity was developed in 50 ml TBS containing 20 mg of DAB, 15 mg of $CoCl_2$ (Sigma, St. Louis) and 50 μl of H_2O_2.

In situ hybridization

Tumors dissected from nude mice were placed in a scintillation vial containing 20 ml of an ice-cold fixative solution composed of 0.2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The vial was put in a plastic measuring cup. Ice-cold water was poured into the cup until the total volume was 200 ml. Then, the cup with the vial was placed in the microwave oven (Bio Rad, Tokyo) and irradiated under 60% power at 10˚C, for 3 min. The fixative solution was then replaced with 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The tissues were irradiated again and then allowed to stand for 2 h. After rinsing well in 0.1 M sodium phosphate buffer, pH 7.2, the tissues were dehydrated by sequential immersion in an up-graded ethanol series and embedded in polyester wax. Serial sections of 4 μm thickness were made and adhered on Vectabond (Vector Laboratories, Burlingame) coated glass slides. The slides were air-dried and stored at 4˚C until use. After dewaxing by immersion in a down-graded ethanol series, they were incubated for 15 min in 0.5 M HCl at RT, then washed three times with PBS for 2 min at RT and incubated with 50% formamide in 2 × SSC for 30 min at 60˚C. Sections were equilibrated in proteinase K buffer (50 mM Tris-HCl, pH 7.5, containing 2 mM $CaCl_2$) at 37˚C for 10 min and then digested with 4 μg/ml of proteinase K (Merck, Dermastadt) in proteinase K buffer for 15 min at 37˚C. After rinsing in PBS, sections were treated with glycine in PBS (2 mg/ml) for 30 sec and fixed with 4% paraformaldehyde in PBS for 3 min at RT. After brief rinsing in PBS, they were again treated with the glycine solution for 5 min, rinsed briefly in PBS, incubated with 1 mM levamisole in dd-H_2O for 5 min at RT and equilibrated in 0.1 M triethanolamine solution, pH 8.0, for 5 min prior to the acetylation. They were acetylated for 5 min with 0.1 M triethanolamine solution, pH 8.0, containing 0.25% acetic anhydrate and 1 mM EDTA at RT, and treated with fresh solution for 10 min at RT. After brief rinsing in PBS, sections were dehydrated by immersing in the up-graded ethanol series and dried. Digoxigenin (DIG)-riboprobes of mouse TN were made using the method described previously (Tsukamoto et al., 1991). Hybridization was carried out as described, but with some modifications. Briefly, hybridization buffer composed of 50% formamide, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 600 mM NaCl, 10 mM DTT, 1 × Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrolidone, 0.02% BSA), 0.25% SDS, 10% dextran sulfate and 200 μg/ml *E.Coli* tRNA was heated at 85˚C for 10 min. The RNA probes were added to this hybridization buffer and heated at 85˚C for 2 min. This probe solution (2 ng/ml)was applied to the serial sections. Hybridization was carried out overnight at 45˚C in the moist chamber. Sections were rinsed in 4 × SSC for 15 min at 42˚C , washed in 2 × SSC 50% formamide at 42˚C for 15 min, then incubated in 1 × TE pH 7.5, 0.5 M NaCl (1 × STE) at 37˚C for 15 min. To remove the nonspecifically attached RNA probes, sections were treated with 10 μg/ml RNase in 1 × STE at 37˚C for 30 min. Thereafter, sections were washed thoroughly in 1 × STE at 37˚C for 15 min, then in 2 × SSC at 42˚C for 15 min twice. In situ hybridization signals were detected according to the protocol furnished with the DIG detection kit (Boehringer Mannheim Yamanouchi, Tokyo). After detection, sections were mounted with coverslips using crystal mount (Biomeda, Costercity).

RESULTS

RCB-1 is specific for human TN

As indicated in Fig. 1, western blot analysis showed that TNs produced by HT1080 and primary mouse and chick embryonic fibroblasts were detected by anti-TN polyclonal antibody (lanes A, B and C). Human TN was detected by RCB-1 (lane D) but mouse and chick TNs were not recognized (lanes E and F). Thus, this is positively demonstrating the availability of RCB-1 for specific staining of human TN.

Cancer cells produce TN when co-cultured with mesenchyme

The results of western blot analysis of various culture media using RCB-1 monoclonal antibody are summarized in Table 1. Both A431 and MCF7 cells produced little TN in vitro. However, when these cells were cultured on the newly prepared feeder layers of embryonic dermis or mammary fat pad precursor tissue, they could secrete detectable amounts of TN into the media (Fig. 2). Tissues from 16-day-old embryos were observed to be most capable (lane B). Mesenchymal cells from mouse neonates (lane D) and adults (lane E) did not have the capacity to induce TN in either A431 or MCF7 cells. None of the established cell lines listed in Table 1 could induce TN in these cells either. Although

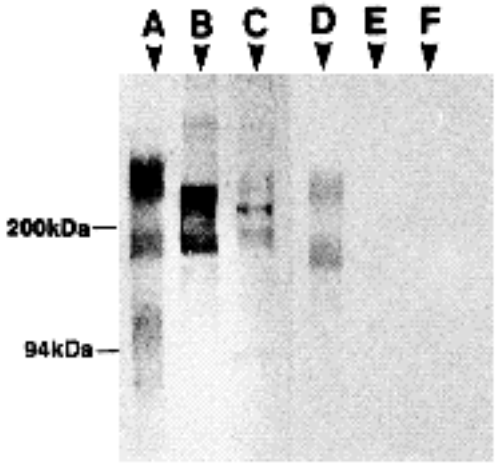

Fig. 1. Specificity of RCB-1 by western blot analysis. Human (lane A), mouse (lane B) and chick (lane C) TNs were detected by anti-TN polyclonal antibody. RCB-1 monoclonal antibody detected human TN (lane D) but not mouse (lane E) and chick lane F) TNs.

Table 1. TN production by A431 and MCF7 cells cultured on various feeder cells or with their conditioned media from feeder cells

	On feeder cells				With conditioned media from feeder cells			
	A431		MCF7		A431		MCF7	
Feeder cells	L	H	L	H	L	H	L	H
Non[1]	−	−	−	−	−	−	−	−
Other cell lines[2]	−	−	−	−	−	−	−	−
Mouse dermis								
14 d.p.c.	+	+	+	+	+	+	+	+
16 d.p.c.	++	++	++	++	++	++	++	++
18 d.p.c.	+	+	+	+	+	+	+	+
New born	−	−	−	−	−	−	−	−
Adult[3]	−	−	−	−	−	−	−	−
Mouse mammary fat pad								
14 d.p.c.	+	+	+/−	+/−	+/−	+	+/−	+/−
16 d.p.c.	++	++	++	++	+	+	+	+
18 d.p.c.	+/−	+/−	+/−	+/−	+/−	+/−	−	−
New born	−	−	−	−	−	−	−	−
Pregnant[4]	−	−	−	−	−	−	−	−
Cleared[5]	−	−	−	−	−	−	−	−
Chick embryonic fibroblasts	++	++	++	++	++	++	++	++

[1]TN was detected neither in culture media nor cell lysates.
[2]Other cell lines include NIH-3T3, ϕ2, STO, 2H6, 3E5, CMT315.
[3]Dermis was taken from adult female mice both carrying and not carrying mammary tumor.
[4]Cells were obtained from pregnant mouse mammary glands containing epithelium as well.
[5]Cells were obtained from no. 4 mammary fat pads of 12-month-old female mice whose mammary epithelia were removed 3 weeks after birth.
L, low band (190 kDa); H, high band (250 kDa); d.p.c., days post coitum.

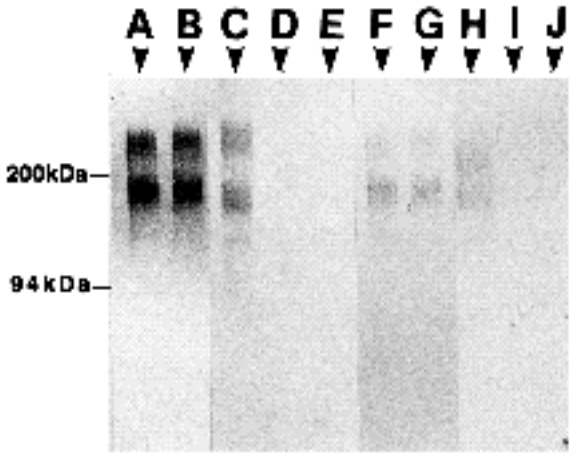

Fig. 2. Western blot analysis of TN induction in A431 cells by co-culture with mouse dermal cells. TN is expressed by co-culture with 14- (A), 16- (B) and 18- (C) days post coitum (d.p.c.) dermal cells but is not expressed with new born (D) and adult (E) dermal cells. Lanes F-J are the results of the conditioned media from 14- (F), 16- (G), 18- (H) d.p.c., new born (I) and adult (J) dermal cells.

mixed cultures of epithelium and mesenchyme, such as 2H6 + 3E5 and NIH3T3 + CMT315, were used for the feeder, the induction of human TN was unsuccessful. The TN molecules produced by A431 and MCF7 in all cases were composed of two major bands, 250 kDa and 190 kDa (Fig. 2).

TN induction in cancer cells by co-culture with mesenchyme is mediated by soluble factor(s)

The conditioned media of various type of cultured cells described above were used to investigate whether they are capable of inducing TN in A431 and MCF7 cells. As shown in Table 1, both embryonic feeder cells and their conditioned media were effective in TN production. A431 and MCF7 cells could produce TN in response to soluble factor(s) from the embryonic cell cultures without direct attachment to the embryonic cells (Fig. 2, F-H).

Dermis induces TN production in A431 cells in vivo

Twenty four hours after transplantation, A431 cells formed a solid mass with a necrotic center at the site of injection and confined to the mouse dermis. This mass increased in volume in an irregular form by making nodular outgrowths during the next two or three days. At five days and afterwards, the A431 cells continued to proliferate, forming a number of cancer nests and invading the surrounding tissues. The size of the cancers was about 2 cm in diameter by 30 days after injection.

Twenty four hours after injection, a heavily deposited mouse TN was immunohistochemically shown in the stroma surrounding the mass of A431 cells (Fig. 3a). Positive staining of TN was also detected in the near papillary dermis and in the basement membranes of hair follicles. This pattern was observed throughout all stages of tumor growth (Fig. 3d and g). In contrast, the location of human TN was different from that in the mouse. One day after injection, human TN was detected as weakly positive, discontinuous lines surrounding the cancer cell mass (Fig. 3b). When the cancer cells proliferate rapidly by nodular outgrowth into the adjacent tissues at 3 days after the injection, the staining of human TN was more pronounced (Fig. 3e). Double staining using RCB-1 and anti-collagen IV or anti-laminin indicated that TN produced by A431 cells deposits in the basement membrane (Fig. 4). At five days and afterwards, immunoreactive human TN was observed in the basement membranes of tumor nests, particularly

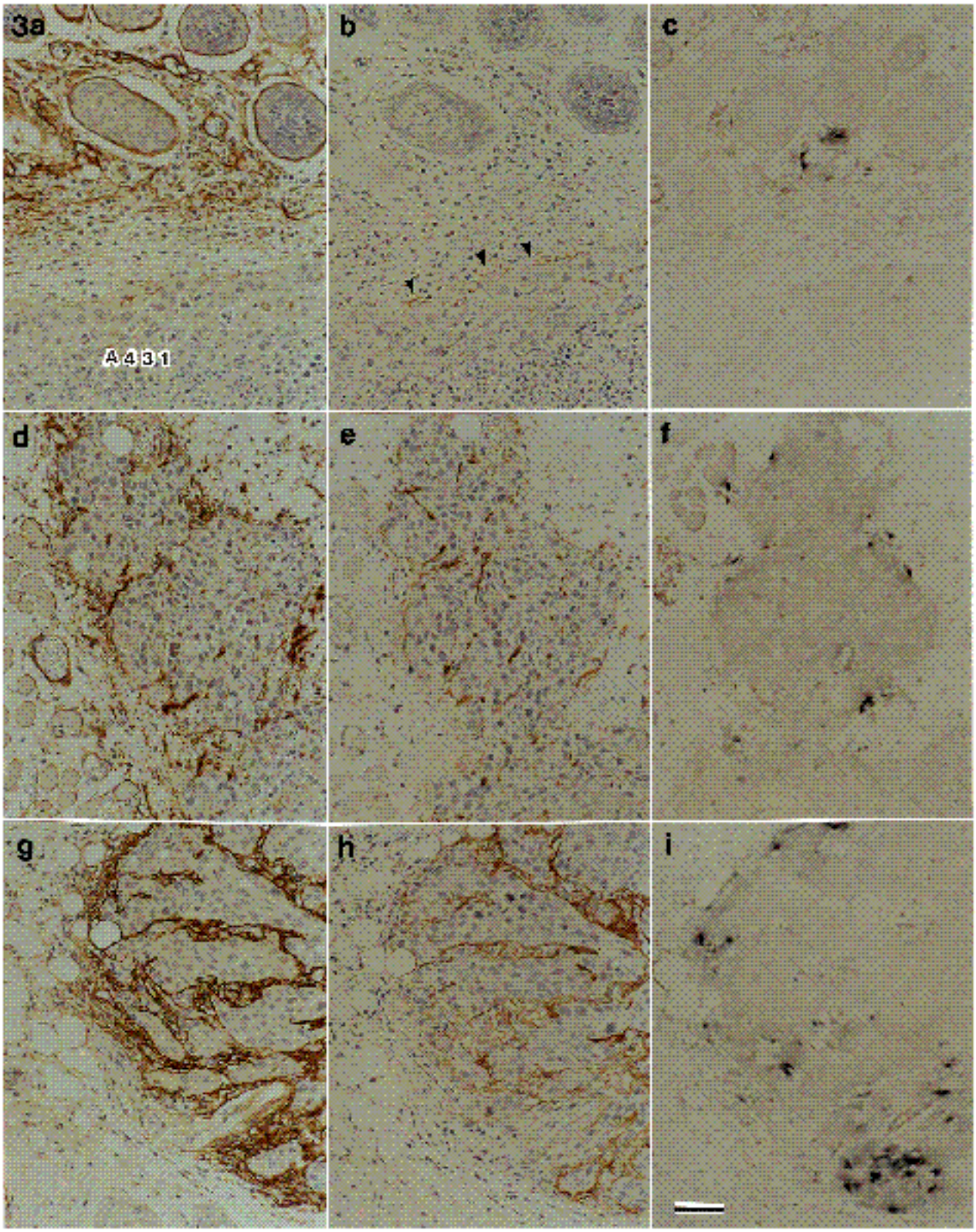

Fig. 3. Expression of TN and TNmRNA in A431 tumors by immunohistochemistory (a, b, d, e, g, h) and by in situ hybridization (c, f, i). Both mouse and human TNs detected by polyclonal antibody are seen in dermal tissues, vessel walls, basement membranes of hair follicles and the stroma surrounding the cancer nests (a, d and g). Human TN detected by RCB-1 is present in basement membranes of cancer nests and nearby mesenchyme (b, e and h). One day (a-c), 3 days (d-f) and 7 days (g-i) after injection. Arrowheads in b show human TN deposition. Bar, 100 μm.

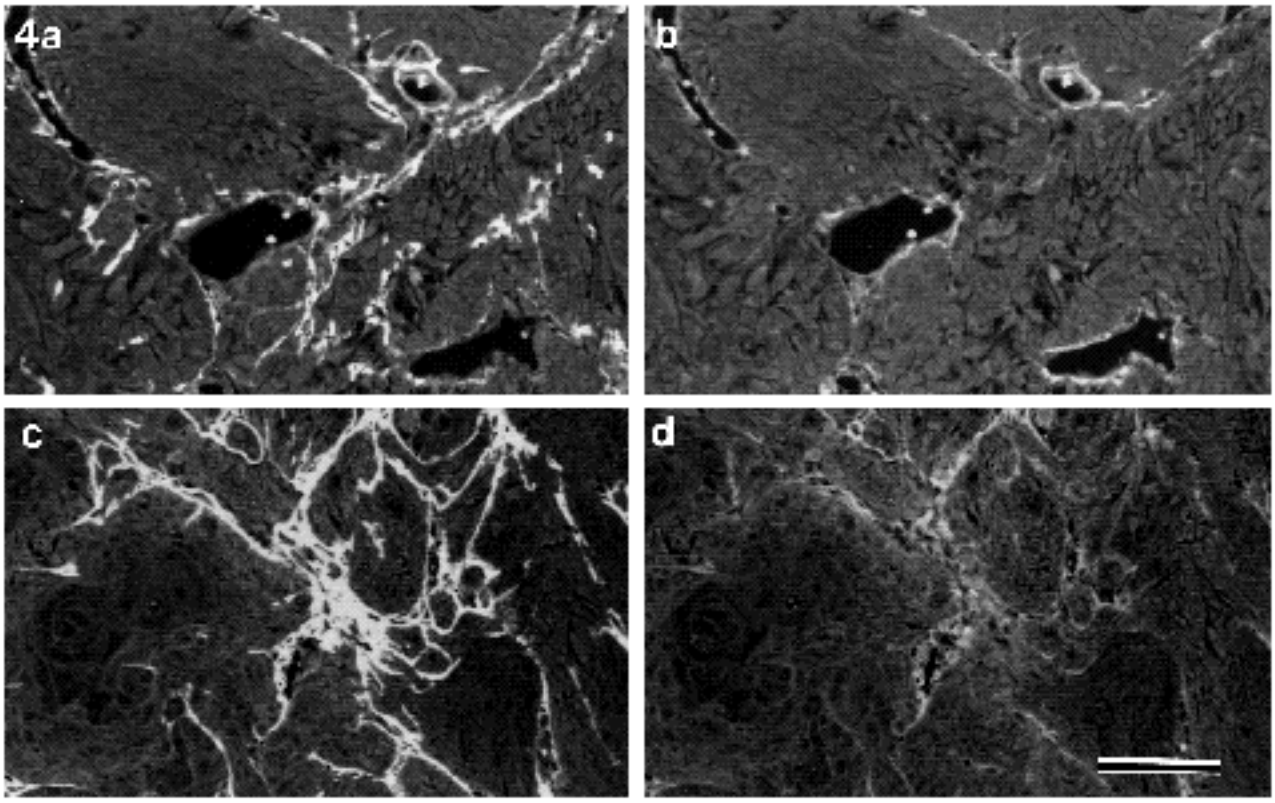

Fig. 4. Double staining of A431 tumor by RCB-1 (a and c) and collagen IV (b) or laminin (d).TN is deposited in basement membrane and nearby mesenchyme. Bar, 100 μm.

between the nests (Fig. 3h). Along the periphery, where the neoplastic cells are proliferating, less human TN expression was seen. Human TN was not detected in the cytoplasm of A431 cells at any stage of tumor growth.

By in situ hybridization, at 24 h after injection, TN mRNA was observed in the dermis and a little in A431 cells (Fig. 3c). Expression of TN mRNA was observed in the dermis during the first few days and then restricted to the papillary dermis and to the stroma surrounding tumors. At 3 days and afterwards, increasing numbers of cells with mRNA expression of TN were detected in the peripheral layers of A431 cancer nests adjacent to the mesenchyme where the deposition of human TN was observed (Fig. 3f). At 7 days after injection, TN mRNA was observed in many tumor cells and the adjacent layers (Fig. 3i) and more at 30 days (Fig. 5).

Other mesenchymal tissues can induce TN production in A431

A431 cells gave rise to tumors at the sites of injections in mammary gland fat pad, testis, liver, kidney, spleen, lung and skeletal muscle as well. In all cases, immunoreactive human TN was detected in the basement membranes and in the dense mesenchyme closely surrounding the cancer nests, the same as in the dermis (Fig. 6).

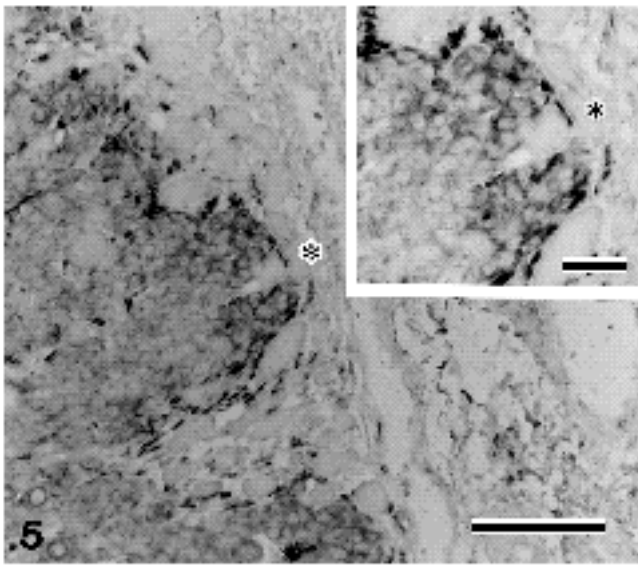

Fig. 5. In situ hybridization of A431 tumor at 30 days after injection. Many A431 cells express TNmRNA. Bar, 100 μm. (Inset) High magnification, bar, 25 μm.

DISCUSSION

Since the tenascin experiment was first reported in 1986 (Chiquet-Ehrismann et al., 1986), a number of other immunohistochemical studies have indicated that TN can be found in the stroma of malignant tumors (Bourdon et al., 1983; Inaguma et al., 1988; Mackie et al., 1989). And,

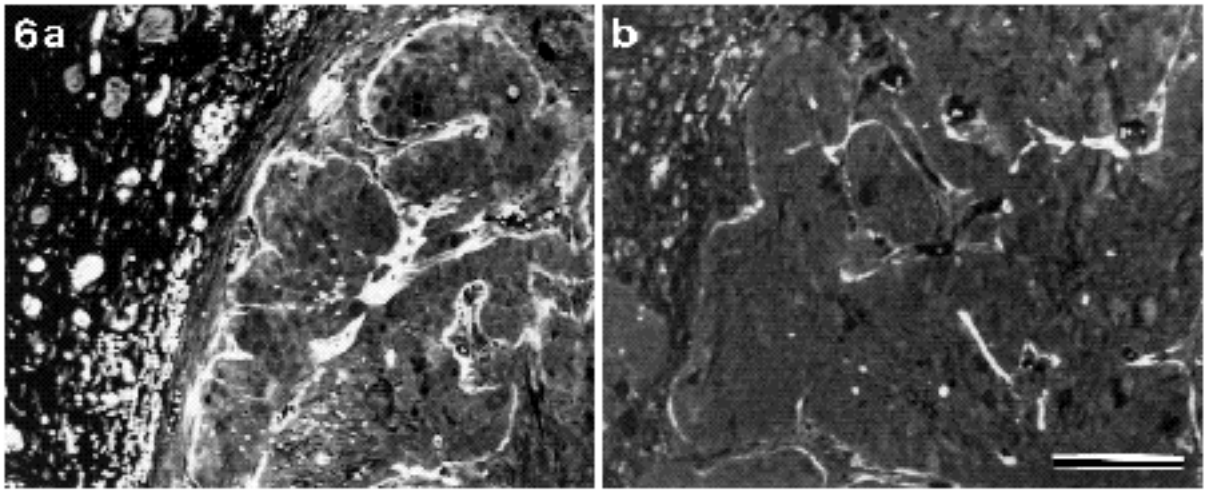

Fig. 6. Immunohistochemistry of TN in A431 tumors developed in mammary fat pad (a) and in skeletal muscle (b). Human TN was detected in the basement membranes and nearby mesenchyme. Bar, 100 μm.

it has not been shown that human epithelial cancer cells can produce TN by themselves, with the exception of glioma (Weller et al., 1991) and melanoma cells (Herlyn et al., 1991). Thus, it has been generally accepted that the cellular source of TN is mesenchyme such as fibroblasts (Chiquet-Ehrismann et al., 1986), smooth muscles (Daniloff et al., 1989) and glias (Tucker and McKay, 1991), but not epithelium. This is the first indication of TN production by epithelial cancer cells and its deposition in the basement membrane and nearby mesenchyme. In the present study, heterotypic combinations of human epithelial cancer cells and mouse mesenchyme were used for in vivo and in vitro experiments. When human epithelial cancer cells were injected into nude mice, TN mRNA expression was observed in the dermis near the injection site during the first few days. This transient expression of TN mRNA in the dermis probably occurs by inflammatory reaction during wound healing (Mackie et al., 1988). Surprisingly, epithelial cancer cells which produce no TN in vitro, could produce TN in the presence of mesenchymal cells in vivo. A faint positive signal of TN mRNA was first demonstrated in cancer cells at one day by in situ hybridization. Positive cells increased in number with tumor development. These unexpected findings led us to speculate that the epithelium produces TN in response to inductive interactions with the surrounding mesenchyme, and secretes it quickly to deposit it in the basement membrane and nearby mesenchyme which then probably affects the epithelium in an autocrine-paracrine fashion.

To verify what induces TN production in the non TN-producing human epithelial cancer cells, these cells were co-cultured with various heterospecific mesenchymal cells. It was found that mouse embryonic mesenchyme was the most effective for TN induction in human epithelial cancer cells. Although chick embryonic fibroblasts were also effective beyond species, but established cell lines, whether from embryos, fresh cultures from newborn or from adult animals, had no effects. On the other hand, conditioned media taken from embryonic mesenchyme could also induce TN production, whereas media from others also had no effect. These results clearly indicate that embryonic mesenchyme releases one or more soluble factor(s) that induces TN production in epithelium. From our preliminary experiments to investigate this soluble factor, conditioned medium lost the capacity if it was stored for 7 days at 4˚C. Of interest, the same effect were reproduced by conditioned medium from non effective mesenchyme, such as newborn or adult mesenchymes, when these mesenchyme cultures were wounded (data not shown). Detailed characterization of these factors is now in process. Recently, a paper has been published reporting the stronger expression of TN in fetal wound than in adult wound in mice (Whitby and Ferguson, 1991). Although the cellular origin of TN production and the mechanism of TN induction have not been clearly demonstrated in the healing wound, it can be speculated that embryonic mesenchyme will provide a more favorable environment for TN production than adult mesenchyme.

As reviewed by Chiquet-Ehrismann (1990), several biological functions of TN have been proposed. Of these, inhibition of cell attachment mediated by fibronectin, seems to be currently favored (Chiquet-Ehrismann et al., 1988; Friedlander et al., 1988). If TN acts on the detachment of cell adhesion mediated by fibronectin in vivo, the detached cells may be provide the suitable microenvironment for their outgrowth. As shown in the chronological observation, TN expression during tumorigenesis is probably explained as follows: during the first few days injected cancer cells led to the construction of several nests, when the amount of human TN expressed was less at the site of cancer cell rearrangement. In growing tumors five days after the injection and beyond, strong staining of human TN was observed in basement membranes particularly between the nests, but negative or very weak staining at the periphery. TN derived from the surrounding mesenchyme may be facilitated for the wound healing process, while TN from epithelial cancer cells may be involved in the tumor development, such as epithelial morphogenesis and subsequent proliferation. Therefore, it is possible to speculate that the function of TN from the epithelial cancer cells differs from the mesenchymal TN.

Our previous studies have demonstrated that embryonic mammary fat pad precursor tissue can support normal organogenesis (Sakakura et al., 1987). This ability was decreased in older embryos and was entirely lost postnatally. Considering this and the present findings together, we propose that TN from epithelium is a key molecule in determining subsequent development and is induced by specific embryonic mesenchyme which is normally present during embryonic stages and reappears in adult mesenchyme as a result of inflammatory processes.

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